



Pergamon

Tetrahedron Letters 41 (2000) 1523–1526

TETRAHEDRON
LETTERS

An efficient method for the synthesis of 2'-*O*-modified nucleosides via double alkylation using cyclic sulfates

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Received 1 December 1999; accepted 14 December 1999

Abstract

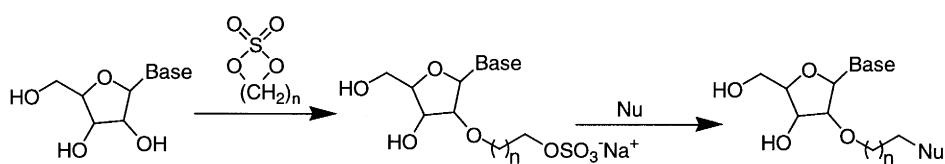
The alkylation of *N*-3-benzyloxymethyl-5-methyluridine with 1,3,2-dioxathiolane 2,2-dioxide or 1,3,2-dioxathiane 2,2-dioxide resulted in a 2'-*O* versus 3'-*O* selectivity of 3:1, respectively. The resulting product has a built-in sulfate leaving group at the terminal end of an ethyl or propyl carbon chain, which can be displaced with sulfur and nitrogen nucleophiles to produce modifications at the 2'-*O* or 3'-*O* positions. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: cyclic sulfate; alkylation; 2'-modified nucleosides.

2'-*O* Modifications of ribonucleotides have shown the ability to enhance the antisense properties of oligonucleotides (e.g., nuclease resistance and binding affinity for the target RNA) (Scheme 1).^{1–3} Several 2'-*O*-aminoalkyl and 2'-*O*-alkylthioalkyl groups have demonstrated very favorable antisense properties.⁴ In order to create an efficient synthesis of 2'-*O*-modified oligonucleotides, one needs a facile method to produce the corresponding monomers. Alkylation of the 2'-*O* position using a five- or six-membered cyclic sulfate followed by nucleophilic displacement would create a modified nucleoside in only two or three synthetic steps. There has been an appreciable amount of work reported on the use of cyclic sulfates^{5–7} in general organic synthetic transformations.⁸ However, their use in novel nucleoside synthesis is a totally unexplored area. In view of the fact that the five- and six-membered cyclic sulfates (namely, 1,3,2-dioxathiolane 2,2-dioxide and 1,3,2-dioxathiane 2,2-dioxide) **A** and **B** (Scheme 2), respectively, are commercially available, we wanted to exploit their use in modified nucleoside synthesis. As a test case, we decided to synthesize 2'-*O*-(*N,N*-dimethylaminoalkyl) and 2'-*O*-[(methylthio)ethyl] nucleosides (Fig. 1).

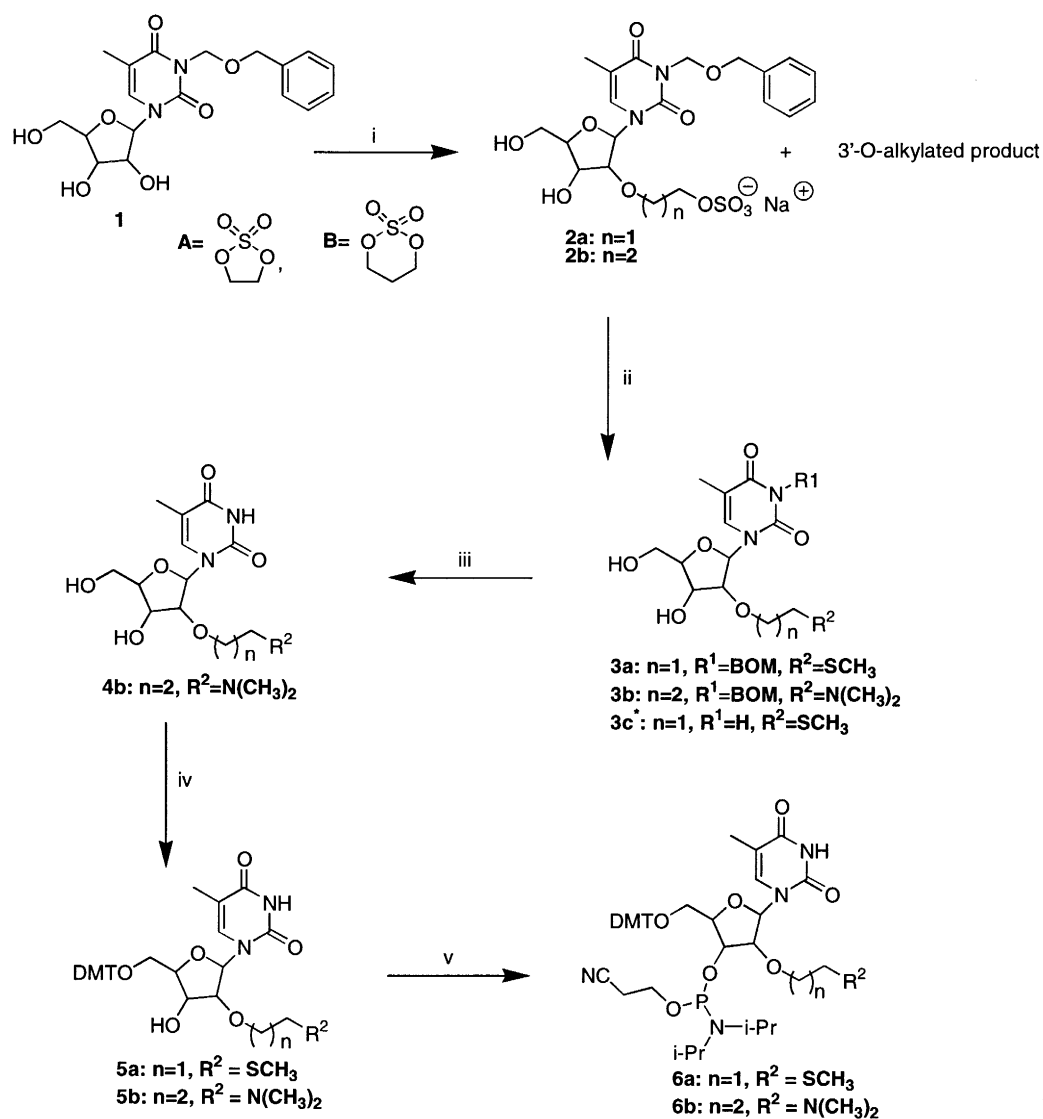
Treatment of the nucleoside **1**⁹ with NaH in DMF at –45°C followed by addition of the cyclic sulfates **A** or **B** afforded compounds **2a** and **2b** in 63 and 50% yields, respectively (Scheme 2). The selectivity for 2' over 3' alkylation was about 3:1 in both cases. Due to the stability of these sodium

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$n = 1$ or 2 ; Nu = nucleophile

Scheme 1.



Scheme 2. Reagents and conditions: (i) NaH, DMF, **A** or **B**, -45°C to rt; (ii) **3a**: NaSCH₃, DMF, 80°C ; **3b**: dimethylamine, THF, autoclave; (iii) Pd(OH), EtOH, AcOH, H₂ at 55 psi; (iv) pyridine, dimethoxytritylchloride; (v) CH₂Cl₂, diisopropylamine tetrazolidate salt, 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphorodiamidite. *The BOM group was deprotected under the same conditions as in (iii) before nucleophilic displacement with NaSCH₃

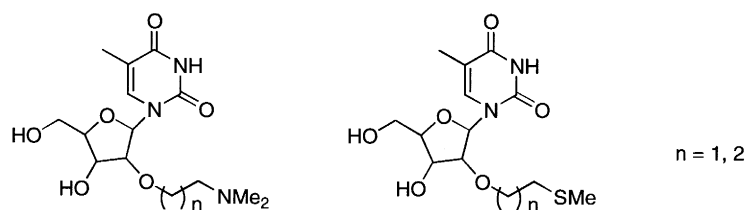


Fig. 1.

sulfate salts as leaving groups, subsequent displacement with nucleophiles required somewhat vigorous reaction conditions. In the case of dimethylamine as a nucleophile an autoclave was used, while in the case of sodium methylmercaptide as a nucleophile, reflux temperatures were needed to obtain nucleophilic displacement of the sulfates to give compounds **3a** and **3b** in moderate to good yields. Intramolecular displacement of sulfate salts by nucleophiles has been reported, but to our knowledge intermolecular displacement has not been reported.^{7,8} Reductive cleavage of the *N*-3-benzyloxymethyl (BOM) protecting group using catalytic hydrogenation over a palladium hydroxide catalyst proceeded normally for compound **3b** to give the 2'-modified nucleoside **4b**¹⁰ in 67% yield. This compound was then protected at the 5'-hydroxyl with a dimethoxytrityl (DMT) group to give compound **5b** (56%) which was then converted to the phosphoramidite **6b** (65%).

In the case of methylthio substitution of the cyclic sulfate, the *N*-3-BOM group of compound **3a** proved to be more difficult to deprotect due to poisoning of the palladium hydroxide catalyst by the sulfide functionality. Therefore, the BOM group of the sulfate derivative **2a** was removed via catalytic hydrogenation over a palladium hydroxide catalyst (product not purified) before nucleophilic displacement with sodium methylmercaptide to give compound **3c** (product not purified). The 5'-hydroxyl group of the crude compound **3c** was converted to the DMT-protected compound (**5a**)¹¹ in 34% yield from **2a**. Conversion of **5a** into the 3'-phosphoramidite **6a** proceeded in 63% yield.

In conclusion, we have found that alkylation of *N*-3-benzyloxymethyl-5-methyluridine with the five- and six-membered cyclic sulfates and subsequent nucleophilic displacement of the sulfate salt leaving group is an efficient, facile method to produce 2'-*O*-modified 5-methyluridine nucleoside monomers for incorporation into oligonucleotides. We have used this methodology to synthesize nucleosides and their phosphoramidites containing 2'-*O*-(*N,N*-dimethylaminopropyl) and 2'-*O*-[(methylthio)ethyl] substituents. The synthesis and evaluation of the properties of the modified oligonucleotides containing these modifications are in progress and will be reported in due course.

Acknowledgements

We are grateful to Robert H. Springer for largescale synthesis of Compound **1**.

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10. Compound **4b**: $^1\text{H NMR}$ ($\text{DMSO-}d_6$) δ 1.65 (bs, 2H), 1.78 (s, 3H), 2.17 (s, H), 2.29 (m, 2H), 3.56 (m, 4H), 3.88 (m, 2H), 4.17 (t, 1H), 4.86 (bs, 1H), 5.19 (bs, 1H), 5.84 (d, 1H), 7.80 (s, 1H), 10.24 (bs, 1H). Anal. calcd for $\text{C}_{15}\text{H}_{25}\text{N}_3\text{O}_6 + 0.25 \text{ mol H}_2\text{O}$: C, 51.79; H, 7.39; N, 12.08. Found: C, 51.94; H, 7.40; N, 11.80. LRMS (ES) $[\text{MH}^+]$ m/z calcd: 344. Found: 344.
11. Compound **5a**: $^1\text{H NMR}$ (CDCl_3) δ 1.42 (s, 3H), 2.03 (s, 3H), 2.74 (m, 2H), 3.13 (d, 1H), 3.46 (dd, 2H), 3.81 (bs, 7H), 4.13 (m, 2H), 4.48 (m, 1H), 5.97 (s, 1H), 6.83 (d, 2H), 7.29 (m, 13H), 7.68 (s, 1H), 8.19 (bs, 1H). Anal. calcd for $\text{C}_{34}\text{H}_{38}\text{N}_2\text{O}_8\text{S} + 1.0 \text{ mol H}_2\text{O}$: C, 62.56; H, 6.18; N, 4.29. Found: C, 62.87; H, 6.00; N, 4.10. LRMS (ES) $[\text{MH}^+]$ m/z calcd: 633. Found: 633.